

Minigene as a novel regulatory element in the toxin-antitoxin systems

Barbara Kędzierska ^{1,*}, Katarzyna Potrykus ¹

Table S1. Plasmids used in this work.

Plasmid	Description	Source
pRW225	RK2-based low copy number vector; enables cloning promoter fragment carrying own translation signals in fusion to lacZ gene between EcoRI and HindIII; Tet ^R	[24]
pRW_ATGmg1	A derivative of pRW225 with paxe promoter sequence up to the ATG 1 amplified with primers 29 and 141; ATG acts as a start codon for lacZ reporter gene.	This work
pRW_GTG	A derivative of pRW225 with paxe promoter sequence up to the GTG amplified with primers 29 and 203; GTG acts as a start codon for lacZ reporter gene.	This work
pRW_ATGmg2	A derivative of pRW225 with paxe promoter sequence up to the ATG 2 amplified with primers 29 and 142; ATG acts as a start codon for lacZ reporter gene.	This work
pRW_ATGtxe	A derivative of pRW225 with paxe promoter sequence up to the ATG txe amplified with primers 29 and 143; ATG acts as a start codon for lacZ reporter gene.	This work
pRW_ATGtxe_ATGmg1mut	A derivative of pRW_ATGtxe where ATG 1 was mutated to ACG with primers 174 and 175	This work
pRW_ATGtxe_GTG/SDmut	A derivative of pRW_ATGtxe where GTG was mutated to CTG with primers 204 and 205	This work
pRW_ATGtxe_ATGmg2mut	A derivative of pRW_ATGtxe where ATG 2 was mutated to ACG with primers 98 and 140	This work
pRW_ATGtxe_mg2mut	A derivative of pRW_ATGtxe where ATG and TAA of mg2 were mutated to ACG and TTA with primers 178 and 179	This work
pRW_ATGtxe_mg1,2mut	A derivative of pRW_ATGtxe_mg2mut where ATG 1 was mutated to ACG with primers 174 and 175	This work
pRW_ATGtxe_w/o mg1,2	A derivative of pRW_ATGtxe where a sequence with minigenes was deleted with primers 143 and 211	This work
pRW_ATGtxe-1	A derivative of pRW_ATGtxe where the distance between ATG 2 and ATG txe was changed by -1 with primers 143 and 212 (deletion of T)	This work
pRW_ATGtxe-2	A derivative of pRW_ATGtxe where the distance between ATG 2 and ATG txe was changed by -2 with primers 143 and 214 (deletion of TC)	This work
pRW_ATGtxe+1	A derivative of pRW_ATGtxe where the distance between ATG 2 and ATG txe was changed by +1 with primers 143 and 207 (insertion of C)	This work
pRW_ATGtxe+2	A derivative of pRW_ATGtxe where the distance between ATG 2 and ATG txe was changed by +2 with primers 143 and 209 (insertion of TC)	This work
pRW_ATGtxe+3	A derivative of pRW_ATGtxe where the distance between ATG 2 and ATG txe was changed by +3 with	This work

	primers 143 and 216 (insertion of CTC)	
pRW_ATGtxe+4	A derivative of pRW_ATGtxe where the distance between ATG 2 and ATG txe was changed by +4 with primers 143 and 218 (insertion of TCTC)	This work
pRW_ATGtxe+5	A derivative of pRW_ATGtxe where the distance between ATG 2 and ATG txe was changed by +5 with primers 143 and 220 (insertion of CTCTC)	This work
pRW_ATGtxe+6	A derivative of pRW_ATGtxe where the distance between ATG 2 and ATG txe was changed by +6 with primers 143 and 222 (insertion of TCTCTC)	This work
pRW_ATGtxe-1_mg2mut	A derivative of pRW_ATGtxe-1 with the mg2 mutated with primers 178 and 179	This work
pRW_ATGtxe-2mg2mut	A derivative of pRW_ATGtxe-2 with the mg2 mutated with primers 178 and 179	This work
pRW_ATGtxe+1_mg2mut	A derivative of pRW_ATGtxe+1 with the mg2 mutated with primers 178 and 179	This work
pRW_ATGtxe+2_mg2mut	A derivative of pRW_ATGtxe+2 with the mg2 mutated with primers 178 and 179	This work
pRW_ATGtxe+3_mg2mut	A derivative of pRW_ATGtxe+3 with the mg2 mutated with primers 178 and 179	This work
pRW_ATGtxe+4_mg2mut	A derivative of pRW_ATGtxe+4 with the mg2 mutated with primers 178 and 179	This work
pRW_ATGtxe+5_mg2mut	A derivative of pRW_ATGtxe+5 with the mg2 mutated with primers 178 and 179	This work
pRW_ATGtxe+6_mg2mut	A derivative of pRW_ATGtxe+6 with the mg2 mutated with primers 178 and 179	This work
pRW_ATGtxe-1_mg2mut_mg1mut	A derivative of pRW_ATGtxe-1_mg2mut with the mg1 mutated with primers 174 and 175	This work
pRW_ATGtxe+2_mg2mut_mg1mut	A derivative of pRW_ATGtxe+2_mg2mut with the mg1 mutated with primers 174 and 175	This work
pRW_ATGtxe-1_mg2mut_GTGmut	A derivative of pRW_ATGtxe-1_mg2mut with the GTG mutated with primers 204 and 205	This work
pRW_ATGtxe+2_mg2mut_GTGmut	A derivative of pRW_ATGtxe+2_mg2mut with the GTG mutated with primers 204 and 205	This work
pBAD33	Arabinose-inducible expression vector, Cm ^R	[47]
pBAD33mg2	A derivative of pBAD33 with the fragment containing minigene 2 amplified with primers 143 and 198, inserted under control of p _{BAD} promoter between PstI and HindIII restriction sites	This work
pBAD33mg1,2	A derivative of pBAD33 with the fragment containing minigenes 1 and 2 amplified with primers 143 and 197, inserted under control of p _{BAD} promoter between PstI and HindIII restriction sites	This work

Table S2. Oligonucleotides used in this study. Restriction enzyme sites and mutated positions are underlined.

Name	Sequence (5'-3')	Description
29	CGCGGGAATTC <u>CTAGAAATAAATAAG</u> GGGT	Forward primer containing EcoRI site for amplification of paxe promoter fragments to clone to pRW225
141	GCAAAGCTTCATCTCCTCGACG	Reverse primer containing HindIII site for amplification of a fragment with ATG mg1 fused to lacZ to clone to pRW225
203	GCAAAGCTTCACCTTTGGAGAATTG	Reverse primer containing HindIII site for amplification of a fragment with GTG fused to lacZ to clone to pRW225
142	GCAAAGCTTCATGTGTTTTAAATGC	Reverse primer containing HindIII site for amplification of a fragment with ATG mg2 fused to lacZ to clone to pRW225
143	GCAAAGCTTCATCAGATTCAACCTCG	Reverse primer containing HindIII site for amplification of a fragment with ATG txe fused to lacZ to clone to pRW225
174	GTCGAGGAGACGAACAATTCTC	Forward primer for mutagenesis of ATG mg1 (ATG→ACG)
175	GAGAATTGTTCTCTCCTCGAC	Reverse primer for mutagenesis of ATG mg1 (ATG→ACG)
204	CTCCAAAGCTGCATTAAAC	Forward primer for mutagenesis of GTG/SD (GTG→CTG)
205	GTTTAAATGCAGCTTTGGAG	Reverse primer for mutagenesis of GTG/SD (GTG→CTG)
98	CATTTAAAACACACGACTTAATC	Forward primer for mutagenesis of ATG mg2 (ATG→ACG)
140	GATTAAGTCGTGTGTTTTAAATG	Reverse primer for mutagenesis of ATG mg2 (ATG→ACG)
179	ATTTAAAACACACGACCTTATCGAGG TTG	Forward primer for mutagenesis of ATG and STOP codons of mg2 (ATG→ACG and TAA→TTA)
178	CAACCTCGATAAGGTCGTGTGTTTTA AAT	Reverse primer for mutagenesis of ATG and STOP codons of mg2 (ATG→ACG and TAA→TTA)
211	GCAAAGCTTCATCAGATTCAACCTCG AAAGTGTTCTCAACG	Reverse primer for amplification of paxe promoter fragment to the ATG txe, devoid of minigenes sequence in between
212	GCAAAGCTTCATCAGATTCAACCTCG TTAAGTCATG	Reverse primer for amplification of paxe fragment to ATG txe with a deletion of T
214	GCAAAGCTTCATCAGATTCAACCTCT TAAGTCATG	Reverse primer for amplification of paxe fragment to ATG txe with a deletion of TC
207	GCAAAGCTTCATCAGATTCAACCTCG AGTTAAGTCATG	Reverse primer for amplification of paxe fragment to ATG txe with an insertion of C
209	GCAAAGCTTCATCAGATTCAACCTCG AGATTAAGTCATG	Reverse primer for amplification of paxe fragment to ATG txe with an insertion of TC
216	GCAAAGCTTCATCAGATTCAACCTCG AGAGTTAAGTCATG	Reverse primer for amplification of paxe fragment to ATG txe with an insertion of CTC

218	GCAA <u>AAGCTT</u> CATCAGATTCAACCTCG AGAGATTAAGTCATG	Reverse primer for amplification of paxe fragment to ATG txe with an insertion of TCTC
220	GCAA <u>AAGCTT</u> CATCAGATTCAACCTCG AGAGAGTTAAGTCATG	Reverse primer for amplification of paxe fragment to ATG txe with an insertion of CTCTC
222	GCAA <u>AAGCTT</u> CATCAGATTCAACCTCG AGAGAGATTAAGTCATG	Reverse primer for amplification of paxe fragment to ATG txe with an insertion of TCTCTC
213	GCAA <u>AAGCTT</u> CATCAGATTCAACCTCG TAAGGTCGTGTG	Reverse primer for amplification of paxe fragment to ATG txe with a deletion of T and mutation of mg2
215	GCAA <u>AAGCTT</u> CATCAGATTCAACCTCT AAGGTCGTGTG	Reverse primer for amplification of paxe fragment to ATG txe with a deletion of TC and mutation of mg2
208	GCAA <u>AAGCTT</u> CATCAGATTCAACCTCG AGTAAGGTCGTGTG	Reverse primer for amplification of paxe fragment to ATG txe with an insertion of C and mutation of mg2
210	GCAA <u>AAGCTT</u> CATCAGATTCAACCTCG AGATAAGGTCGTGTG	Reverse primer for amplification of paxe fragment to ATG txe with an insertion of TC and mutation of mg2
217	GCAA <u>AAGCTT</u> CATCAGATTCAACCTCG AGAGTAAGGTCGTGTG	Reverse primer for amplification of paxe fragment to ATG txe with an insertion of CTC and mutation of mg2
219	GCAA <u>AAGCTT</u> CATCAGATTCAACCTCG AGAGATAAGGTCGTGTG	Reverse primer for amplification of paxe fragment to ATG txe with an insertion of TCTC and mutation of mg2
221	GCAA <u>AAGCTT</u> CATCAGATTCAACCTCG AGAGAGTAAGGTCGTGTG	Reverse primer for amplification of paxe fragment to ATG txe with an insertion of CTCTC and mutation of mg2
223	GCAA <u>AAGCTT</u> CATCAGATTCAACCTCG AGAGAGATAAGGTCGTGTG	Reverse primer for amplification of paxe fragment to ATG txe with an insertion of TCTCTC and mutation of mg2
197	TAGT <u>CTGCAGT</u> GGAATAATTCGTCG AGG	Forward primer for amplification of a fragment containing minigenes 1 and 2 sequence, to clone to pBAD33, with PstI site
198	TAGT <u>CTGCAGT</u> GGAACAATTCTCCAAA GGTGC	Forward primer for amplification of a fragment containing minigene 2 sequence, to clone to pBAD33, with PstI site

Table S3. A list of bacterial strains tested for the presence of two-ORF minigenes. In the indicated TA families two-ORF minigenes were not found.

Bacterial strain	TA family
<i>Escherichia coli</i> CFT073	yefM-yoeB
<i>Escherichia coli</i> str. K-12 substr. MG1655	yefM-yoeB
<i>Lactobacillus rhamnosus</i> Lc 705	yefM-yoeB
<i>Streptomyces avermitilis</i> MA-4680	yefM-yoeB
<i>Streptomyces coelicolor</i> A3(2)	yefM-yoeB
<i>Agrobacterium tumefaciens</i> str. C58 Atu0935	relBE
<i>Agrobacterium tumefaciens</i> str. C58 Atu0675	relBE
<i>Agrobacterium vitis</i> S4	relBE
<i>Aliivibrio salmonicida</i> LFI1238	relBE
<i>Archaeoglobus fulgidus</i> DSM 4304 AF1094	relBE
<i>Archaeoglobus fulgidus</i> DSM 4304 AF1081	relBE
<i>Archaeoglobus fulgidus</i> DSM 4304 AF1076	relBE
<i>Bacteroides fragilis</i> YCH46	relBE
<i>Bartonella henselae</i> str. Houston-1 BH07070	relBE
<i>Bifidobacterium longum</i> subsp. <i>infantis</i> ATCC 15697	relBE
<i>Bradyrhizobium diazoefficiens</i> USDA 110	relBE
<i>Brucella melitensis</i> str. 16M	relBE
<i>Brucella suis</i> 1330	relBE
<i>Campylobacter jejuni</i> RM1221	relBE
<i>Candidatus Protochlamydia amoebophila</i> UWE25 pc0996	relBE
<i>Candidatus Protochlamydia amoebophila</i> UWE25 pc1913	relBE
<i>Caulobacter crescentus</i> CB15 CC_2514	relBE
<i>Caulobacter crescentus</i> CB15 CC_0803	relBE
<i>Desulfovibrio vulgaris</i> str. Hildenborough	relBE
<i>Geobacillus kaustophilus</i> HTA426	relBE
<i>Geobacter sulfurreducens</i> PCA GSU2438	relBE
<i>Gloeobacter violaceus</i> PCC 7421 glr4239	relBE
<i>Gloeobacter violaceus</i> PCC 7421 gsr3527	relBE
<i>Gloeobacter violaceus</i> PCC 7421 gsl1825	relBE
<i>Helicobacter pylori</i> 26695 HP0895	relBE
<i>Idiomarina loihiensis</i> L2TR IL1162	relBE
<i>Legionella pneumophila</i> str. Lens lpl1587	relBE
<i>Methanococcus maripaludis</i> S2 MMP0477	relBE
<i>Methanosarcina mazei</i> Go1 MM_2681	relBE
<i>Methylococcus capsulatus</i> str. Bath MCA2652	relBE
<i>Mycobacterium bovis</i> AF2122/97 Mb3392	relBE
<i>Mycobacterium tuberculosis</i> CDC1551 MT3465	relBE
<i>Mycobacterium tuberculosis</i> H37Rv Rv3357	relBE
<i>Nitrosomonas europaea</i> ATCC 19718 NE1998	relBE
<i>Nitrosomonas europaea</i> ATCC 19718 NE1583	relBE
<i>Nitrosomonas europaea</i> ATCC 19718 NE0712	relBE
<i>Nostoc</i> sp. PCC 7120 (<i>Anabaena</i> sp. PCC 7120) all4408	relBE
<i>Pectobacterium atrosepticum</i> SCRI1043 ECA0444	relBE
<i>Pectobacterium atrosepticum</i> SCRI1043 ECA0325	relBE
<i>Photobacterium profundum</i> SS9 PBPRB1200	relBE
<i>Photobacterium profundum</i> SS9 PBPRB0955	relBE
<i>Pseudomonas aeruginosa</i> PA1 PA1S_26100	relBE
<i>Pseudomonas aeruginosa</i> PA1 PA1S_26070	relBE

<i>Pseudomonas aeruginosa</i> PAO1 PA0125	relBE
<i>Pseudomonas putida</i> KT2440 PP_2499	relBE
<i>Pseudomonas putida</i> KT2440 PP_1268	relBE
<i>Pseudomonas syringae</i> pv. <i>Tomato</i> str. DC3000 PSPTO_5388	relBE
<i>Pseudomonas syringae</i> pv. <i>Tomato</i> str. DC3000 PSPTO_3676	relBE
<i>Shewanella oneidensis</i> MR-1 SO_4642	relBE
<i>Sinorhizobium meliloti</i> 1021 SMc00693	relBE
<i>Streptococcus agalactiae</i> NEM316 gbs0471	relBE
<i>Streptococcus mutans</i> UA159 SMU_895	relBE
<i>Streptococcus pneumoniae</i> R6 spr1104	relBE
<i>Streptococcus pneumoniae</i> R6 spr0252	relBE
<i>Streptococcus pneumoniae</i> TIGR4 SP_0275	relBE
<i>Streptomyces cattleya</i> NRRL 8057 = DSM 46488 SCATT_39270	relBE
<i>Sulfolobus tokodaii</i> str. 7 STS035	relBE
<i>Synechocystis</i> sp. PCC 6803 SYNGTS_2983	relBE
<i>Synechocystis</i> sp. PCC 6803 ssr1114	relBE
<i>Thauera</i> sp. MZ1T Tmz1t_3767	relBE
<i>Thermoanaerobacter tengcongensis</i> MB4	relBE
<i>Thermococcus kodakarensis</i> KOD1 TK0966	relBE
<i>Thermococcus kodakarensis</i> KOD1 TK0792	relBE
<i>Treponema denticola</i> ATCC 35405 TDE1979	relBE
<i>Vibrio cholerae</i> O1 biovar <i>El Tor</i> str. N16961 VCA0349	relBE
<i>Vibrio cholerae</i> O1 biovar <i>El Tor</i> str. N16961 VCA0504	relBE
<i>Clostridium acetobutylicum</i> ATCC 824 CA_C0493	mazEF
<i>Deinococcus radiodurans</i> R1 DR_0661	mazEF
<i>Deinococcus radiodurans</i> R1 DR_0416	mazEF
<i>Desulfovibrio vulgaris</i> str. Hildenborough DVU1510	mazEF
<i>Enterococcus faecalis</i> V583 EF3261	mazEF
<i>Escherichia coli</i> O157:H7 str. EDL933 Z5835	mazEF
<i>Escherichia coli</i> O157:H7 str. Sakai ECs5202	mazEF
<i>Geobacter uraniireducens</i> Rf4 Gura_2715	mazEF
<i>Gloeobacter violaceus</i> PCC 7421 gsr4049	mazEF
<i>Gloeobacter violaceus</i> PCC 7421 gsr2287	mazEF
<i>Gloeobacter violaceus</i> PCC 7421 gsr0095	mazEF
<i>Lactobacillus johnsonii</i> NCC 533 LJ0588	mazEF
<i>Leptospira interrogans</i> serovar <i>Copenhageni</i> str. Fiocruz L1 - 130 LIC11192	mazEF
<i>Leptospira interrogans</i> serovar <i>Lai</i> str. 56601 LA_2844	mazEF
<i>Leptospira interrogans</i> serovar <i>Lai</i> str. 56601 LA_1780	mazEF
<i>Methylococcus capsulatus</i> str. Bath MCA3006	mazEF
<i>Mycobacterium bovis</i> AF2122/97 Mb1531	mazEF
<i>Mycobacterium bovis</i> AF2122/97 Mb0679c	mazEF
<i>Mycobacterium bovis</i> AF2122/97 Mb1978c	mazEF
<i>Mycobacterium tuberculosis</i> CDC1551 MT2047	mazEF
<i>Mycobacterium tuberculosis</i> CDC1551 MT1541	mazEF
<i>Mycobacterium tuberculosis</i> CDC1551 MT0689	mazEF
<i>Mycobacterium tuberculosis</i> H37Rv Rv0456B	mazEF
<i>Mycobacterium tuberculosis</i> H37Rv Rv1943c	mazEF
<i>Mycobacterium tuberculosis</i> H37Rv Rv1494	mazEF
<i>Neisseria meningitidis</i> MC58 NMB2037	mazEF
<i>Nitrosomonas europaea</i> ATCC 19718 NE1580	mazEF

<i>Nocardia farcinica</i> IFM 10152 nfa7700	mazEF
<i>Nostoc</i> sp. PCC 7120 (<i>Anabaena</i> sp. PCC 7120) asl3212	mazEF
<i>Nostoc</i> sp. PCC 7120 (<i>Anabaena</i> sp. PCC 7120) asr0757	mazEF
<i>Pectobacterium atrosepticum</i> SCRI1043 ECA0583	mazEF
<i>Photorhabdus luminescens</i> subsp. <i>laumondii</i> TTO1 plu2366	mazEF
<i>Pseudomonas putida</i> KT2440 PP_0770	mazEF
<i>Rhodopseudomonas palustris</i> CGA009 RPA0942	mazEF
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> COL SACOL2059	mazEF
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> MRSA252 SAR2157	mazEF
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> MSSA476 SAS1974	mazEF
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> Mu50 SAV2069	mazEF
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> MW2 MW1993	mazEF
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> N315 SAS067	mazEF
<i>Staphylococcus epidermidis</i> ATCC 12228 SE1673	mazEF
<i>Staphylococcus epidermidis</i> RP62A SERP1682	mazEF
<i>Streptococcus mutans</i> UA159 SMU_172	mazEF
<i>Synechococcus</i> sp. WH 8102 SYNW2380	mazEF
<i>Synechocystis</i> sp. PCC 6803 ssl2245	mazEF
<i>Thermoanaerobacter tengcongensis</i> MB4	mazEF
<i>Thermococcus kodakarensis</i> KOD1 TK1815	mazEF
<i>Brucella melitensis</i> bv. 1 str. 16M BMEI1375	phd-doc
<i>Brucella suis</i> 1330 BR0558	phd-doc
<i>Campylobacter jejuni</i> RM1221 CJE1101	phd-doc
<i>Candidatus Protochlamydia amoebophila</i> UWE25 pc1457	phd-doc
<i>Candidatus Protochlamydia amoebophila</i> UWE25 pc1022	phd-doc
<i>Enterococcus faecalis</i> V583 EF0380	phd-doc
<i>Methanosarcina mazei</i> Go1 MM_0550	phd-doc
<i>Mycobacterium smegmatis</i> str. MC2 155 MSMEG_1277	phd-doc
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Typhimurium</i> str. LT2 STM3559	phd-doc
<i>Streptococcus pneumoniae</i> TIGR4 SP_0888	phd-doc
<i>Streptomyces coelicolor</i> A3(2) SCO5908	phd-doc
<i>Xanthomonas axonopodis</i> pv. <i>citri</i> str. 306 XAC1195	phd-doc
<i>Staphylococcus aureus</i> SACH_a19	phd-doc

P_{axe}
 →
 AAGAAACGTTGAGAACACTTTCTAATAATTACGTCATCGAAAAAATTCGTC
 GAGGAGATGAAACAATTCTCCAAGCTGCATTTAAACACATGACTTAATCGAGGTTGAATCTGATG
 mg1 GTG mg2 txg

Figure S1. Nucleotide sequence of the *txe* leader where deleted fragment is indicated by a strikethrough line – used in the ATGtxe_w/o mg1,2 construct.

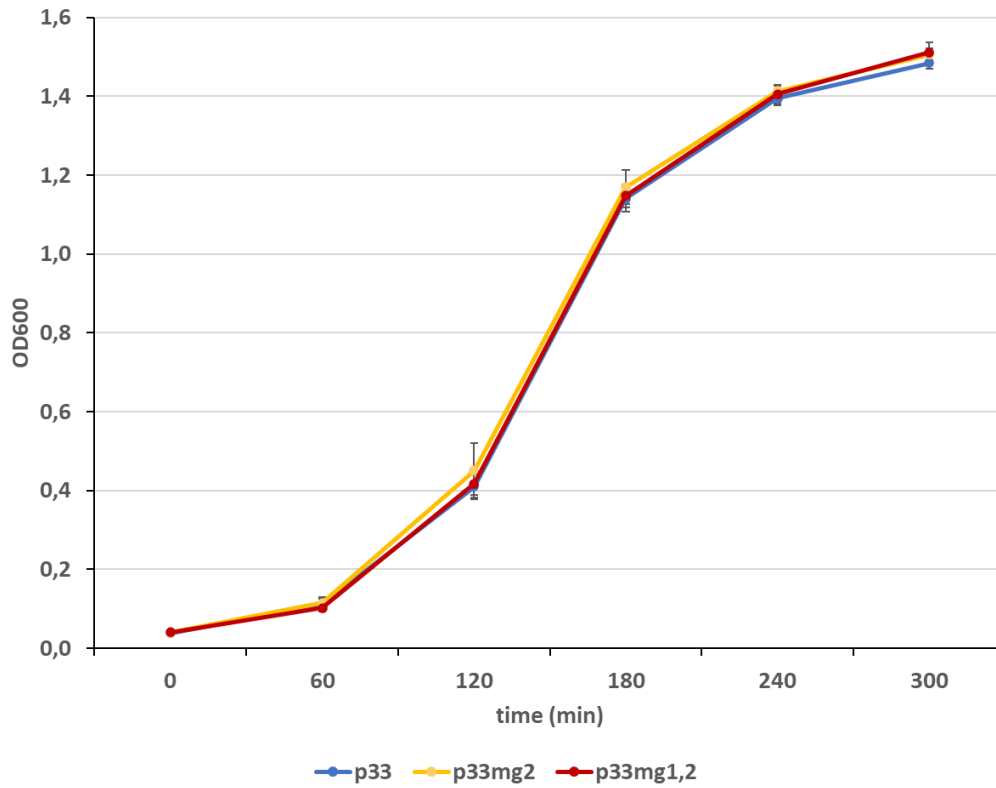


Figure S2. Minigenes encoded in the *txe* leader do not display toxic effect on bacterial cell growth. *E. coli* $\Delta 5\Delta lac$ strain was transformed with pBAD33 or its derivatives, pBAD33_mg2 or pBAD33_mg1,2 bearing minigenes under *p_{BAD}* promoter. Expression of minigenes was induced by addition of 0.2% L-arabinose at a time of inoculation. Absorbance readings at 600 nm were taken at 60 min. intervals. These results are average of five independent experiments, error bars represent standard deviation (S.D.).

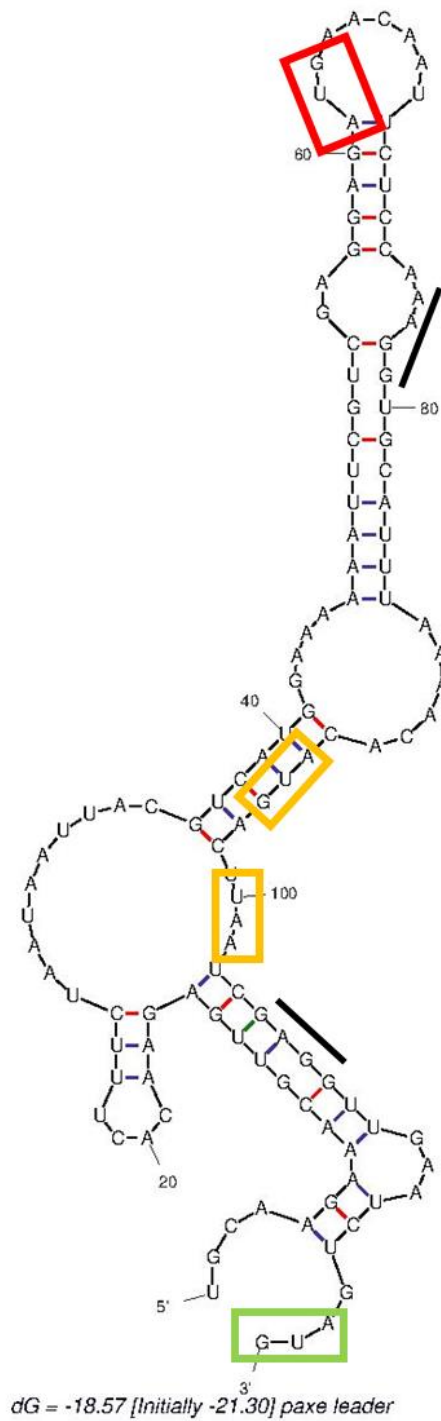


Figure S3. Secondary structure of *txe* leader fragment created with the mFOLD program (<http://mfold.rna.albany.edu/>). No evident strong secondary structures are formed. Start codons of *mg1*, *mg2* and ATG*txe* are in colored boxes, red, yellow and green, respectively. UAA *mg2* stop codon is in yellow box. SD sequences are underlined.